Effect of Polymer End Group on the Formation of Styrene-Maleic Acid Lipid Particles (SMALPs)

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Introduction & Objectives

Membrane proteins (MPs) are the target for **over 70% of pharmaceuticals**.[1] However, to date, MPs account for **less than 2% of all high resolution images** in the entire Protein Structural Database.[2] This is due to **reliance on detergent micelles** to stabilise MPs in aqueous media, often **causing MPs to mis-fold or denature**, meaning structure and function are indeterminable.[3]

SMALP nanodiscs can **incorporate MPs along with native cellular membranes**, offering enhanced stability and improved spectroscopic analysis.[3] Here, the **effects of polymer end group** on SMALP formation are probed to **scope opportunities to functionalise SMA** and refine SMALP applications.

Synthesis

- Commercial polymers are **polydisperse** and have **random** monomer architecture.
- Therefore, **controlled polymerisation methods** (RAFT) were employed to isolate polymeric effects upon nanodisc self assembly.
- **End group lacks attention** in the literature but **represents a chemically unique and targetable unit** - in the case of RAFT, a thiol susceptible to established **click chemistry**.
- Therefore, the **hydrophobic S_3C_{12}** end group was **exchanged for a hydrophilic CN** group.

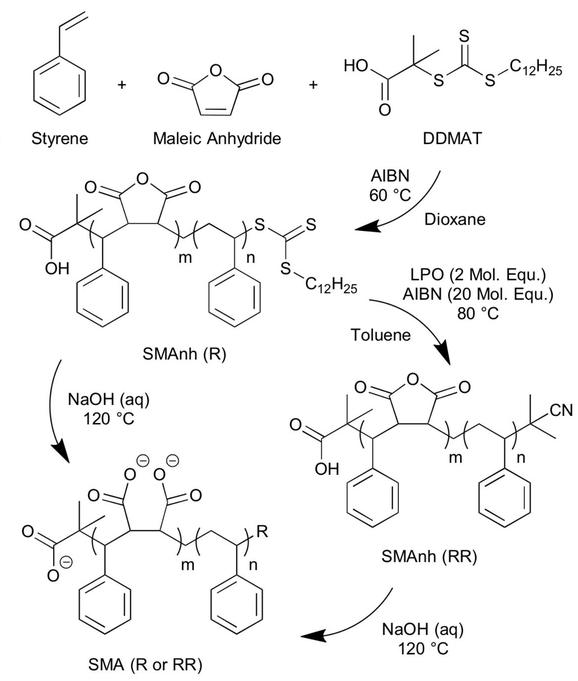
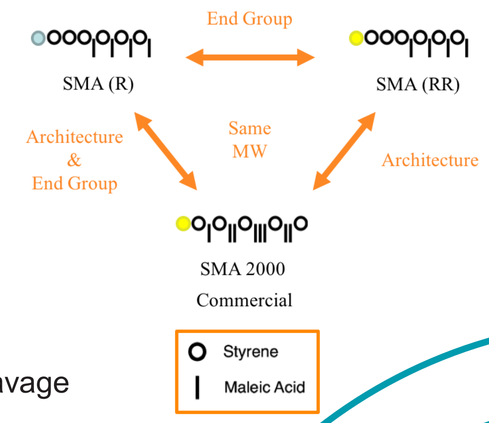


Fig 1. *RAFT synthesis of styrene-maleic acid copolymer (SMAnh R) subsequent end group exchange to SMAnh RR, and hydrolysis of both polymers to acid form (SMA R or RR).*

Polymer Characterisation

RAFT induces **block architecture** and hydrophobic **end groups** unseen in commercial SMA.



...Extensive characterisation required:

- ^{1}H NMR = Monomer ratio (2:1)
- ^{13}C NMR = Monomer architecture
- DOSY NMR & UV-vis = End group cleavage
- GPC = Molecular weight

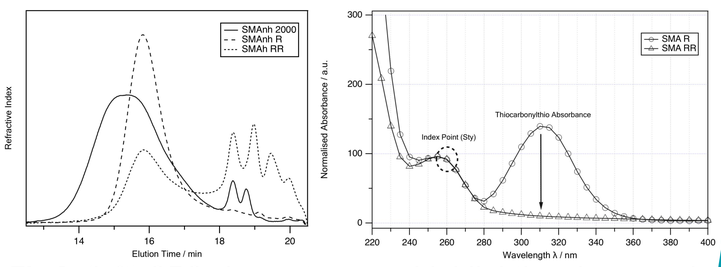
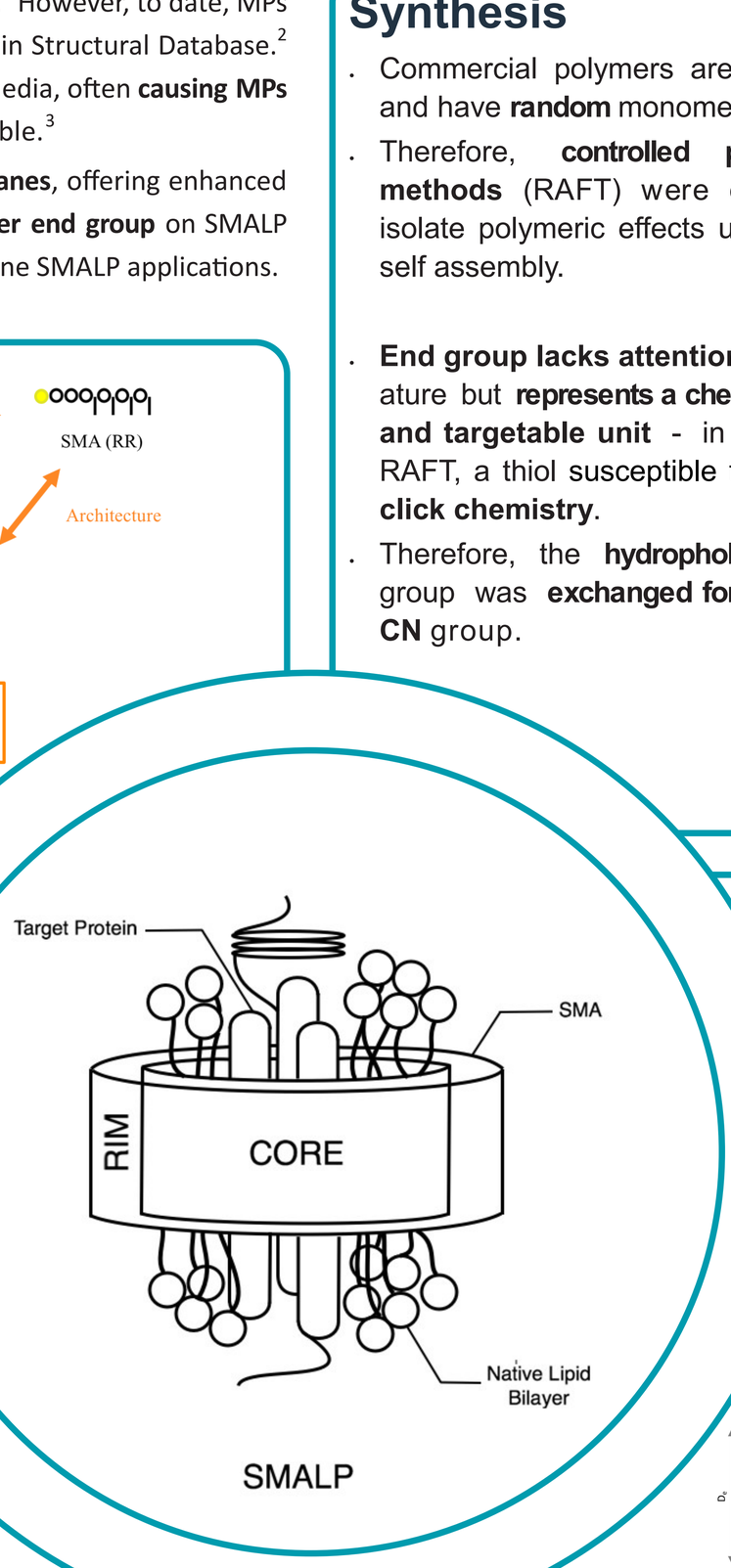
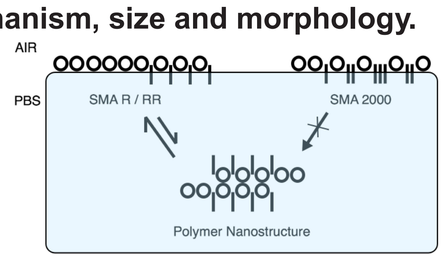


Fig 2. *(left) GPC chromatograms highlighting decreased polydispersity but comparable MW to commercial variant. (right) UV-vis spectra showing removal of S_3C_{12} end group.*



Aggregate Behaviour

Drop tensiometry between SMA solutions and air or dodecane (C_{12}) indicate how **polymer amphiphilicity** couples with polymer end group and architecture. Potential to **predict eventual nanodisc assembly mechanism, size and morphology.**



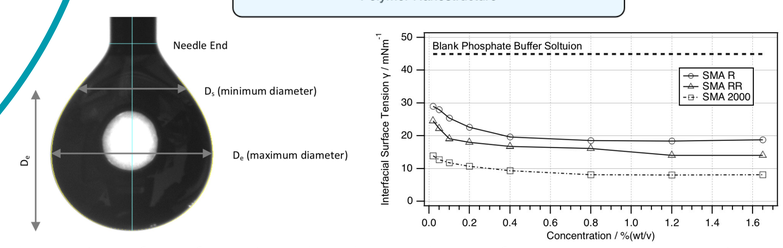


Fig 3. *(top) Schematic of surface tension behaviour of polymer aggregates. (bottom left) Key parameters in drop tensiometry. (bottom right) Surface tension results.*

Small Angle Neutron Scattering

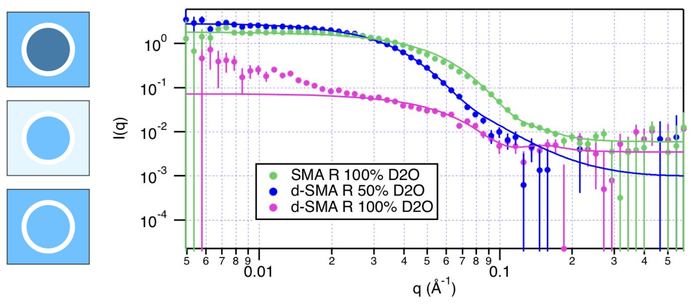


Fig 4. *(left) Schematic of variant solvent contrasts used to isolate aggregate morphology. (right) Scattering data of SMA R aggregates fit to a core shell sphere model.*

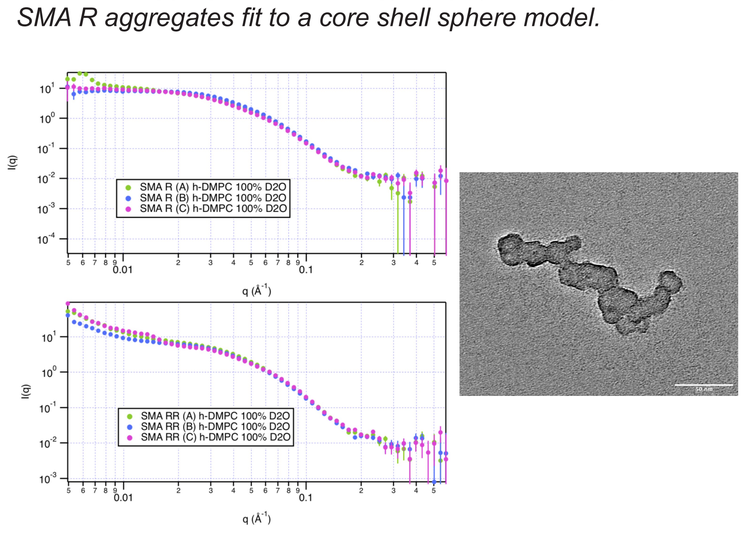


Fig 5. *(left) Scattering of SMA R (top) & SMA RR (bottom) nanodiscs reveal different behaviours between polymers. (right) TEM micrograph of linear SMALP aggregation.*

SANS results show:

- Aggregates have a **core structure** to protect styrene homoblocks from solvent.
- Similarly, in SMALPs, **the homoblock prefers to insert** into the lipid fragment - **inducing swelling** with increasing polymer MW.
- **Hydrophilic CN end groups disrupt this insertion** - instead **causing end-to-end aggregation** of nanodiscs.

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Conclusions & Future Work

- Aggregation behaviour of SMA has the **potential to predict** the polymer's propensity to form SMALPs - **possibly facilitating the discovery** of new polymers for nanodiscs.
- Polarity of polymer end groups can significantly alter nanodisc size and stability:
  - Hydrophobic end groups **allow homoblock insertion** - providing stability but threatening the conservation of MP integrity.
  - Hydrophilic end groups circumvent this - demonstrating **homoblock insertion is not necessary** to form SMALPs.
- Future work to assess the opportunity to **functionalise polymer end groups** to facilitate new applications of SMALPS, such as **lab-on-chip diagnostics**, **magnetic separation** of MPs and **energy harvesting**.

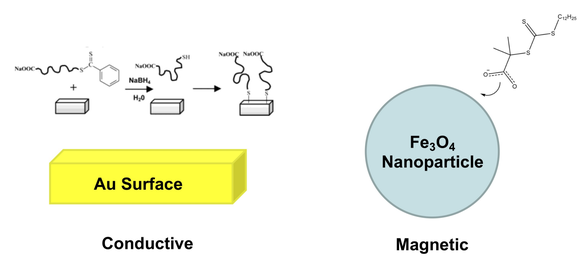

Fig 5. *(left) Gold surface functionalisation with RAFT polymers.[4] (right) Magnetic nanoparticle functionalisation with RAFT agents.[5]*

[1] T. J. Knowles, R. Finka, C. Smith, Y. P. Lin, T. R. Dafforn and M. Overduin, *J. Am. Chem. So.*, 2009, **131**, 7484-7485.

[2] Z. Stroud, S. C. L. Hall and T. R. Dafforn, *Methods*, 2018, **147**, 106-117.

[3] J. M. Dorr, S. Scheidelaar, M. C. Koorengevel, J. J. Dominguez, M. Schafer, C. A. van Walree and J. A. Killian, *Eur. Biophys. J.*, 2016, **45**, 3-21.

[4] B. S. Sumerlin, A. B. Lowe, P. A. Stroud, P. Zhang, M. W. Urban and C. L. McCormick, *Langmuir*, 2003, **19**, 5559-5562.

[5] B. D. White, Master's thesis, University of Bath, 2018.

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